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Research Article

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In Vitro Insulin Resistance of Human Adipocytes Isolated from Subjects with Noninsulin-dependent Diabetes Mellitus

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ABSTRACT To assess possible cellular mechanisms of in vitro insulin resistance in noninsulin-dependent diabetes mellitus (NIDDM), maximum insulin-stimulated glucose transport and utilization and insulin binding were measured in adipocytes isolated from weight-matched normal glycemic subjects and patients with NIDDM. Glucose transport rate was determined by measuring the amount of [U-^{14}C]-D-glucose taken up by incubating adipocytes at trace concentrations of glucose (300 nM), and glucose metabolism by estimating the amount of lactate, CO_2, triglyceride, and total glucose carbons retained in the cells following incubating at 5.5 mM glucose. Insulin binding was measured at 50, 100, and 200 pM [mono^{125}I-*tyrosinyl* A_{14}]insulin. Both maximum insulin-stimulated glucose transport and utilization in adipocytes from diabetic subjects were 40% ($P < 0.01$) and 32% ($P < 0.05$) lower, respectively, than values obtained for subjects with normal glucose tolerance. In addition, the maximum capacity of glucose transport was correlated with the maximum capacity of glucose utilization ($r = 0.81$, $P < 0.001$). Furthermore, fasting plasma glucose concentrations of diabetic subjects were negatively correlated with both maximum insulin-stimulated glucose transport ($r = -0.56$, $P < 0.05$) and glucose utilization ($r = -0.67$, $P < 0.05$). Since basal glucose transport in adipocytes from diabetic subjects was also 33% lower than in adipocytes from normal subjects, there was no change in the relative ability of insulin to stimulate glucose transport. However, there was a 64% decrease in the sensitivity of the glucose transport system to insulin ($P < 0.05$), unrelated to concomitant changes in insulin binding. These results demonstrate that both maximal insulin-stimulated glucose transport and utilization, and the sensitivity of the glucose transport system to insulin, was decreased in adipocytes isolated from subjects with NIDDM. These in vitro defects were associated with impaired glucose metabolism in vivo, consistent with the view that the metabolic alterations observed at the cellular level may contribute to the in vivo insulin resistance of NIDDM.

INTRODUCTION

Although it is clear that patients with noninsulin-dependent diabetes mellitus (NIDDM)[1] are characterized by a decrease in the ability of insulin to stimulate in vivo glucose utilization (1–6), the mechanisms responsible for the "insulin resistance" are just beginning to be understood. Evidence has been published which indicates that the number of insulin receptors in circulating monocytes are reduced in patients with NIDDM (4, 7, 8), but this defect does not appear to totally account for the insulin resistance present in this syndrome (4). As a result, it is likely that changes distal to the binding of insulin also play a role in the insulin resistance of NIDDM. To define putative postreceptor defects, it would be helpful if insulin-sensitive tissues could be used to quantify insulin action in vitro. Liver, muscle, and adipose tissue all qualify in this regard, but only adipose tissue can be obtained relatively easy for this purpose. Furthermore, methods are available that can be used to study in vitro insulin action on adipocytes isolated from man (9, 10). Therefore, in an

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[1] *Abbreviation used in this paper:* NIDDM, noninsulin-dependent diabetes mellitus.

effort to help define the cellular basis for the in vivo insulin resistance of patients with NIDDM, we have determined insulin binding and the ability of insulin to stimulate glucose transport and utilization in adipocytes isolated from obese subjects with either normal glucose tolerance or NIDDM.

METHODS

Chemicals. Hepes buffer (pH 7.4) with albumin was prepared as described previously (11). Albumin (bovine, fraction V) was obtained from Armour Pharmaceuticals, Kankakee, IL; collagenase (type I) from Worthington Biochemical Corp., Freehold, NJ; porcine insulin from Ely Lilly & Co., Indianapolis, IN; glycine-hydrazine buffer (pH 9.2), NAD, lactic acid dehydrogenase, lactate, and osmium tetroxide were all from Sigma Chemical Co., St. Louis, MO; silicone oil from Union Carbide Corp., New York; hyamine hydroxide, [U-^{14}C]-D-glucose (329 mCi/mmol), [1-^{3}H]-L-glucose (10 mCi/mmol), and [U-^{14}C]-D-3-*O*-methylglucose (329 mCi/mmol) was from New England Nuclear, Boston, MA; [mono^{125}I-*Tyr* A_{14}]insulin from Novo International Corp., Copenhagen, Denmark; all other chemicals were of analytical grade. Dulbecco's phosphate-buffered saline was from Gibco Laboratories, Grand Island, NY.

Subjects. 14 patients with NIDDM (8 female, 6 male) and 14 control subjects (obese, normal glycemic, 8 female, 6 male) between 19 and 36 yr old were admitted to the Phoenix Clinical Research Section after giving informed consent. The subjects were Pima/Papago Indians, and clinical data of the subjects are summarized in Table I. All subjects received a weight-maintaining diet comprised of 15% protein, 45% carbohydrate, and 40% fat for at least 3 d before biopsy or oral glucose tolerance test. Individuals were classified using the criteria recommended by the National Diabetes Data Group (12): normal glycemic subjects had fasting plasma glucose concentrations <115 mg/dl, plasma glucose at times <2 h was <200 mg/dl, and 2-h plasma glucose <140 mg/dl; diabetic subjects had fasting plasma glucose ≥140 mg/dl or both 2-h plasma glucose ≥200 mg/dl and either 30- or 60-min plasma glucose ≥200 mg/dl. No subjects were taking medication at the time of the study.

Oral glucose tolerance tests (75 g) were performed within 7 d of the fat biopsy and after at least 3 d on the weight-maintaining diet, and consisted 0, 30, 60, 120, and 180 min time points. Plasma glucose concentration was measured by the glucose oxidase method (13), using a glucose analyzer (Beckman Instruments, Inc., Fullerton, CA), and insulin concentration by radioimmunoassay, using the dextran-coated charcoal separation technique (14).

Preparation of isolated adipocytes. Subjects were fasted for at least 14 h before biopsy. Subcutaneous adipose tissue was removed from the lateral aspect of the hypogastrium, inferior to the umbilicus (McBurney's point). The skin was infiltrated with 2% xylocaine, taking care not to infiltrate subcutaneous tissue. An eliptical incision ~4 cm long was made, and a wedge-shaped sample of subcutaneous fat underlying the incision (5–15 g) dissected.

Isolated adipocytes were prepared by the collagenase method developed by Rodbell (15) in rats, modified for use in human adipocytes by Pedersen et al. (10). Briefly, adipose tissue was washed two times with 10 ml of Delbucco's phosphate-buffered saline at room temperature, and all obvious connective tissue and blood clots removed. The sample was cut into small pieces, and up to 6 g of tissue was added to 6 ml of collagenase solution (Hepes buffer containing 3.5% albumin, 1 mg/ml of collagenase, and 0.55 mM glucose) in 30-ml polyethylene Nalgene bottles. The bottles were shaken at 140 cycles/min at 37°C for 75 min. The cell suspension was passed through 450-μm nylon mesh. The cells were allowed to float for 3 min. The infranatant was removed and 20 ml of fresh buffer containing 5% albumin added. This washing procedure was repeated three times. The cells were passed through nylon mesh a second time, and then concentrated to a 40% lipocrit for final dispersion to vials for incubations.

Cells to be utilized in glucose metabolism studies were washed and reconcentrated to 30% in Krebs-Ringer bicarbonate buffer (pH 7.4) equilibrated with 95% O_2/5% CO_2. Cells were never allowed to float without shaking for >5 min in order to prevent cohesion between cells that causes them to break on later shaking.

Determination of cell size. Isolated adipocytes were fixed with osmium tetroxide, washed, and diluted in saline as described by Cushman and Salans (16). Cells were sized using a Coulter Electronic Cell Counter (model ZB, Coulter Electronics, Inc., Hialeah, FL) with a 400-μm aperture, equipped with logarithmic range-expanded channelyzer. The volume of cells in the 100 channels was then calculated from a known volume in a reference channel. The average volume of cells in all channels were divided by the total number of cells sized. Cell volume was then converted to micrograms lipid per cell, using the correction factor of 0.91 μg lipid/nl cell volume. No clumping of adipocytes or partial digestion of the fat tissue was observed by viewing the fixed adipocytes under a microscope.

Insulin binding. Insulin binding was determined in quadruplicate by incubating a 300-μl suspension of isolated adipocytes (7%) in 5% albumin Hepes buffer in the presence of 0.5 mg/ml bacitracin, 50 pM [mono^{125}I-*Tyr* A_{14}]insulin (0.6–0.9 μCi/pmol) and 0, 50 pM, 150 pM, or 10 μM insulin

TABLE I
Clinical Data of the Subjects

Subjects	n	Age	BMI	Adipocyte size	Fasting plasma glucose	2-h plasma glucose	Fasting insulin level
		yr	*kg/m²*	*μg lipid/cell*	*mg/dl*		*μU/ml*
Control	14	25.2±1.2	41±1	0.82±0.04	91±2	120±5	42±5
Diabetic	14	28.4±1.5	40±2	0.83±0.09	190±17	328±19	39±6
P		NS	NS	NS	<0.0001	<0.0001	NS

All data are expressed as mean±SEM. BMI, body mass index.

as described previously (11). Incubations were carried out for 50 min at 37°C, with constant shaking at 160 times/min, terminated with cold saline, and the cells separated from the medium by centrifugation through oil (11). The cells were collected in a disposable pipet tip and assayed for radioactivity in a auto-γ-counter (Packard Instrument Co., Inc., Downers Grove, IL). The 50, 100, and 200 pM insulin groups were corrected for nonspecific binding by subtracting values obtained in the presence of 10 μM insulin. Even 50 pM insulin is not always a trace concentration (i.e., a concentration at which there is no measurable competition among insulin molecules) due to the much higher affinity of human adipocytes insulin receptors for insulin (10). Therefore, we calculated the fraction bound in units of picoliters per cell or femtoliters per square micrometer from the "Y" intercept of the Scatchard equation as follows. "Bound" insulin per cell or per cell surface area, was calculated and with the "Free" insulin concentrations substituted into the Scatchard equation (Bound/Free = $(-1/K_d)$ (Bound) + Ro/K_d; K_d, dissociation constant; Ro, receptor number). Least squares analysis was utilized to obtain the best fit value of the Y intercept (Ro/K_d). The correlation coefficients were >0.9 except for measurements where Bound/Free was the same for all three insulin concentrations.

Measurement of glucose transport. Conventionally, analogues of glucose, such as 3-*O*-methylglucose, which are transported by the glucose transport system, but not further metabolized, have been used to assess cellular glucose transport. These sugars are taken up by the adipocytes exponentially over time, since net uptake decreases as the extracellular/intracellular concentration difference disappears (17). Obtaining an estimate of the initial rate of uptake has been difficult (18), since the adipocytes have a small water space (19) that is rapidly filled (18). Recently Whitesell and Gliemann (20) solved this problem using a rapid pulse technique. Modification of this method has been successfully applied to human adipocytes by Pedersen and Gliemann (9) and Ciaraldi et al. (21). Although we have also been able to utilize the rapid pulse technique in human adipocytes (22), we have been concerned that the coefficient of variation of quadruplicate samples for this assay, due primarily to errors in the timing, rapid pipeting, and mixing, can be as high as 10–20%. In addition, insulin degradation approximates 16%/60 min at low insulin concentrations using the concentrated cell suspensions (40%) needed for this assay. Although the rapid pulse method is essential for kinetic experiments, it may be more complicated than necessary for simple measurements of transport at different insulin concentrations. Furthermore, this approach necessitates 20 μl packed cells/sample, may require several different time points (some human subjects have very slow basal transport rates), is difficult to perform, and the expense of 3-*O*-methylglucose is considerable.

To avoid these problems, we have developed a new method for determination of cellular glucose transport based on the premise that glucose uptake provides a measurement of glucose transport when studies are carried out at very low glucose concentrations. Specifically, isolated adipocytes (2% lipocrit) were incubated in 500 μl 5% albumin buffer in the presence of 0, 25, 50, 100, 200, 800, and 8,000 pM insulin and trace (300 nM) amounts of 0.1 μCi [U-^{14}C]-D-glucose. The cell suspension was incubated at 37°C for 1 h with continuous shaking at 40 cycles/min. The incubation was terminated by centrifuging a 400-μl aliquot in a 550-μl microfuge tube (23), and the amount of radioactivity associated with the adipocytes (as well as the total radioactivity in the incubation medium) determined by liquid scintillation counting. Under these conditions, ~20% of the adipocyte-associated activity was water soluble, with the remainder being recovered as triglyceride. It should be noted that <2% of the tracer is converted to CO_2. Finally, by the use of an anion exchange resin (24), it was possible to determine that >99% of the radioactivity remaining in the incubation medium was present as glucose. Therefore, glucose metabolites were not escaping from the cells in detectable quantities. Based upon these measurements, it was possible to calculate the glucose transport rate expressed as the glucose clearance rate in fl/cell·s: (clearance = volume in medium·cpm in cells/cpm in medium or, moles/concentration in the medium).

The validation of the method is seen in Fig. 1. The results in Fig. 1 *A* demonstrate that glucose clearance in the absence of insulin was constant up to a glucose concentration of 550 μM. In contrast, insulin-stimulated glucose clearance declined significantly between glucose concentrations of 5–100 μM. This observation was independent of glucose tolerance, and demonstrates that this approach to assessing glucose transport can only be utilized at very low glucose concentrations.

The decline in glucose transport at higher glucose concentrations could be due to the fact that there was increased competition for glucose transporters at higher glucose concentrations in incubation medium. However, this seems unlikely in view of the known characteristics of the glucose transport system in adipocytes. Thus, direct measurements of the kinetics of the glucose transport system indicate that the dissociation constant (K_s) for glucose is ~9 mM (21). Since clearance = V_{max}/K_s + S (substrate concentration), the decrease in clearance between 5 and 550 μM glucose cannot be accounted for by competition among glucose molecules. Thus, the decline in glucose clearance observed at higher glucose concentrations (>5 μM) must be due to the fact that steps in the pathway of glucose utilization, not glucose transport, become rate limiting at these concentrations. Similar changes in the relationship between glucose clearance and incubation medium glucose concentrations have been defined in rat adipocytes.[2]

A direct comparison of both basal and insulin-stimulated glucose transport using 3-*O*-methylglucose (with the rapid pulse method [22]) and the new assay are shown in Fig. 1 *B*. These two measurements are highly correlated ($r = 0.91$, $P < 0.001$) and resulted in an almost one-to-one relationship. The relative stimulation determined with the two methods of measuring transport were also similar. Basal and maximal insulin-stimulated transport rates, as well as the fold stimulation, were also similar, whether measured from trace glucose uptake during a 2-h incubation or from the rapid pulse method in isolated rat adipocytes (23). Thus, the results from the two transport methods are similar. In addition, since the K_s for glucose is higher (9 mM) than that of 3-*O*-methylglucose (6.6 mM) (21), and the apparent V_{max} = clearance·K_s at substrate concentrations much less than the K_s (17), the apparent V_{max} of glucose is as high as that of 3-*O*-methylglucose.

To determine if the close relationship between measurements of glucose and 3-*O*-methylglucose transport are also present under identical incubation conditions, a comparison was made between (10 μM) D-glucose clearance and 3-*O*-methylglucose (10 μM) clearance, both using the rapid pulse method for 10 s as described previously (22). The clearance rates were 156±37 fl/cell·s and 177±28 fl/cell·s, respectively (mean±SEM, $n = 4$). Thus, the relationship between

[2] Gliemann, J., and J. E. Foley. Unpublished observations.

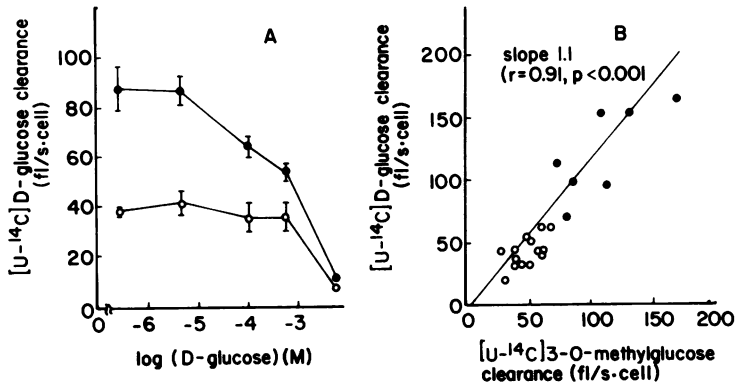

FIGURE 1 (*A*) [U-^{14}C]-D-Glucose clearance as a function of D-glucose concentrations (0.3, 5, 100, and 550 μM and 5.5 mM) in adipocytes isolated from normal glycemic subjects in the presence (●) and absence (○) of 8 nM insulin. Values are means±SD of triplicate determinations. Clearance was determined at 0.3, 5, and 100 μM glucose as described in measurements of trace glucose transport in the Methods. Clearance at 550 and 5,500 μM D-glucose were measured as described previously in the Methods as total glucose utilization. (*B*) Correlation between [U-^{14}C]-D-glucose clearance (0.3 μM) and [U-^{14}C]-3-*O*-methylglucose clearance (10 μM) in the presence (●) and absence (○) of 8 nM insulin. Each point is the mean of triplicate determinations using the new transport method and the rapid pulse method described previously (22).

trace glucose and 3-*O*-methylglucose transport was independent of the time of incubation or the cell concentration. In addition, when glucose clearance using the rapid pulse method was compared with that of the new uptake method at 30, 60, and 120 min, the clearance increased linearly with time (r = 0.99).

Given all of the above considerations, we believe that determination of [U-^{14}C]-D-glucose uptake by adipocytes incubated at tracer glucose concentrations provides a useful alternative method for assessing glucose transport independent of glucose tolerance. The glucose method avoids the timing problems and complexities of the rapid pulse technique, utilizes <50% as many cells per sample as the 3-*O*-methylglucose method, makes it easier to do insulin dose-response experiments and greatly lowers isotope cost (<10%). The intraassay coefficient of variation is 5–10% (Fig. 2) with the glucose method. The glucose method does not obviate the 3-*O*-methylglucose method. The 3-*O*-methylglucose method is the only method available for kinetic studies. Furthermore, in the hands of very skilled and practiced individuals, the coefficient of variation of the 3-*O*-methylglucose assay can be as good as that found with the glucose assay.

Insulin sensitivity. The concentration of insulin resulting in a half-maximal transport rate (ED_{50}) was determined from linear regression of transport rate vs. the log of the insulin concentrations at 25, 50, 100, 200, and 800 (if <80% of value at 8,000) pM insulin. The correlation coefficient between these two variables was >0.95 in all experiments.

Glucose metabolism. Total glucose utilization was determined by incubating cells with [U-^{14}C]glucose in the presence of 0.55 or 5.5 mM glucose. Total glucose uptake was calculated by a summation of moles of glucose transported, released as CO_2, which were retained in the cells and which were released in the incubation medium as lactate. Nonlipid intracellular intermediates were calculated from the difference between glucose carbons retained in the cell and glucose incorporation into triglyceride.

Glucose oxidation and glucose incorporation into triglyceride. Isolated adipocytes (4%) were incubated in 1 ml Krebs-Ringer bicarbonate buffer (pH 7.4) equilibrated with 95% O_2 and 5% CO_2 containing 5% albumin, 0.5 μCi [U-^{14}C]glucose and 5.5 mM glucose in the presence of 8 nM insulin at 37°C for 2 h with a continuous shaking at 40 cycles/min. The incubation was terminated by adding 300 μl of 8 N sulfuric acid. $^{14}CO_2$ production was measured as described previously by Gliemann (25) and glucose incorporation into triglyceride was determined by the method described by Dole and Meinertz (26). Under these conditions, the total glucose utilization by the cells was linear for at least 3 h.

Measurements of glucose carbons retained in adipocytes and released from adipocytes as lactate. Isolated adipocytes (4%) were incubated in 500 μl Krebs-Ringer bicarbonate buffer (pH 7.4) equilibrated with 95% O_2 and 5% CO_2 containing 5% albumin, 0.25 μCi [U-^{14}C]glucose, and 0.55 or 5.5 mM glucose in the presence of 8 nM insulin at 37°C for 2 h with a continuous shaking at 40 cycles/min. The incubation was terminated following the oil-flotation method as described previously (23). Following separation by centrifugation, the packed cell layer was used for the measurement of the glucose retained in the cells, and the incubation medium below the oil layer was used for the measurement of lactate. Lactate was determined by incubating 200 μl of the medium with NAD and lactic dehydrogenase in glycine and hydrazine buffer (pH 9.2), using a pyruvate and lactate assay kit (Sigma Chemical Co.) (27).

RESULTS

Fig. 2 depicts the ability of varying amounts of insulin to stimulate glucose uptake of adipocytes isolated from one control subject and one patient with NIDDM. In this instance it is clear that maximal insulin-stimulated glucose clearance was lower in adipocytes of the patient with NIDDM, and insulin sensitivity (ED_{50}) was also reduced.

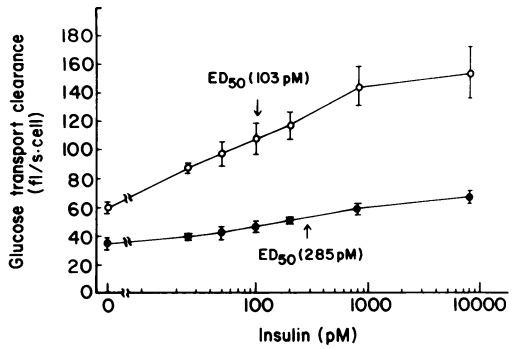

FIGURE 2 Insulin dose-response curve of glucose transport in adipocytes isolated from an obese normal glycemic control (○) and an obese NIDDM subject (●). Values are mean±SD of triplicate determinations. ED_{50} were calculated as described in the Methods.

The values for these two measurements for each individual subject were averaged, and the results are seen in Fig. 3. The data in Fig. 3 *A* indicate that both basal ($P < 0.05$) and maximal insulin-stimulated ($P < 0.01$) glucose clearance were decreased in adipocytes from patients with NIDDM. However, the relative ability of insulin to maximally increase glucose transport over basal values was similar in the two groups.

The results in Fig. 3 *B* demonstrate that the ED_{50} of adipocytes from the NIDDM group was significantly increased ($P < 0.05$). In other words, adipocytes from patients with NIDDM were less sensitive to the ability of insulin to maximally stimulate glucose transport.

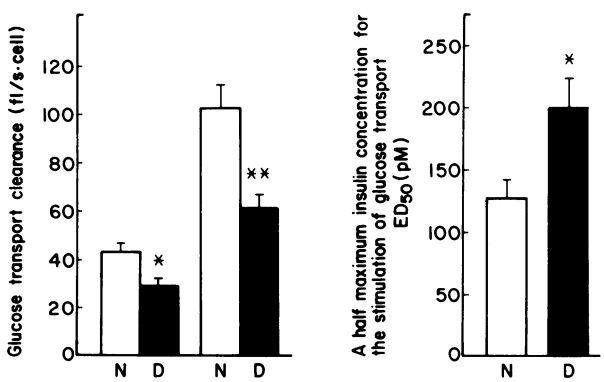

FIGURE 3 Glucose transport in obese normal glycemic subjects (N) and obese NIDDM subjects (D) in the absence (basal) and presence of 8 nM insulin. Data are expressed as the mean±SEM of 14 subjects. $^{*}P < 0.05$, $^{**}P < 0.01$ (*left* panel). A half-maximum insulin concentration for the stimulation of glucose transport (ED_{50}) in obese normal glycemic subjects (N) and obese NIDDM subjects (D). Values are the mean±SEM of 14 subjects. $^{*}P < 0.05$ (*right* panel).

The maximum insulin-stimulated glucose metabolism in the presence of 5.5 mM glucose is depicted in Table II and demonstrates that maximum glucose utilization was reduced by 32% in adipocytes from patients with NIDDM ($P < 0.05$). On the other hand, relative conversion of glucose to its metabolites was similar in adipocytes isolated from both subject groups. The percentage of glucose converted to lactate was higher than the values that could have been predicted by previous studies with rat epididymal fat (28), but is consistent with recent studies by Crandell et al. (29) in rat subcutaneous fat cells. Greater than 99% of the triglyceride fraction was triglyceride-glycerol (data not shown).

The results in Fig. 3 *A* indicated that maximal insulin-stimulated glucose transport by adipocytes from patients with NIDDM was reduced by ~40%, and a similar reduction in insulin-stimulated glucose metabolism was seen in Table II. These results suggest that these two effects might be correlated, and the data in Fig. 4 demonstrates that this was the case ($r = 0.81$, $P < 0.001$).

The effect of NIDDM on insulin binding can be seen in Table III, and it is clear that insulin binding neither per cell nor per cell surface area was significantly decreased in the subjects with NIDDM as compared with the obese normal glycemic controls. Thus, changes in insulin binding cannot account for the 67% decrease in sensitivity of the glucose transport system to insulin. Both binding per cell and per cell surface area are shown, since both expressions have been shown to be potentially important in relating insulin binding to sensitivity of the transport system to insulin (7, 23).

The data in Fig. 5 represent an effort to assess the possible relationship between the observed changes in insulin-stimulated glucose transport and metabolism to the metabolic defects in patients with NIDDM. Thus, the results in Fig. 5 *A* and *B* demonstrate that the degree of fasting hyperglycemia was significantly negatively correlated with both maximum glucose transport ($r = -0.56$, $P < 0.05$) and glucose utilization ($r = -0.67$, $P < 0.05$) in subjects with NIDDM. These correlations are consistent with the view that changes in maximum insulin stimulation of glucose transport and metabolism may play a role in the insulin resistance of diabetic subjects, and this inference is further supported by the observation that plasma glucose concentrations 2 h after a 75-g oral glucose challenge were also negatively correlated with maximum insulin-stimulated glucose transport ($r = -0.57$, $P < 0.05$) and glucose utilization ($r = -0.69$, $P < 0.05$). In contrast, decreased fasting insulin values were not significantly correlated with either maximum insulin-stimulated glucose transport or glucose utilization in subjects with NIDDM.

TABLE II

Maximum Insulin-stimulated Glucose Metabolism in the Presence of 5.5 mM Glucose

Subjects	n	Total glucose utilization	Percentage of total glucose utilization			
			CO_2	Triglyceride	Lactate	Nonlipid intracellular metabolites
		amol/cell/s				
Control	12	136±12	3±0.4	18±2	62±1	17±2
Diabetic	11	93±11*	4±0.6	18±1	62±2	16±2

All data are expressed as mean±SEM.

* $P < 0.05$ by nonpaired t test (compared with the values of normal subjects).

DISCUSSION

The results of this investigation demonstrate that both maximal insulin-stimulated glucose transport and metabolism were reduced in adipocytes isolated from patients with NIDDM as compared with values of subjects with normal glucose tolerance. Although the diabetic patients were overweight, the control group was equally heavy, and the differences noted cannot be attributed to obesity. Somewhat similar observations as to changes in glucose metabolism of adipocytes from patients with NIDDM have recently been reported by three laboratories (30–32),[3] as well as our own (33), suggesting that the findings are not unique to our patient population. Given these observations, it seems reasonable to conclude that alterations in the regulation by insulin of adipocyte glucose metabolism exist in patients with NIDDM.

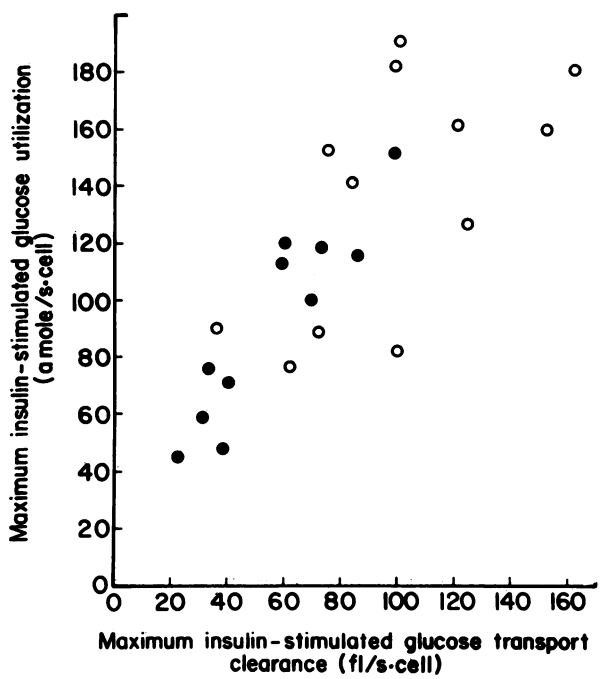

FIGURE 4 Correlation between maximum insulin-stimulated glucose utilization and maximum insulin-stimulated glucose transport in adipocytes isolated from obese normal glycemic subjects (○) and subjects with NIDDM (●). ($r = 0.81$, $P < 0.001$).

Documentation of defects in insulin-stimulated glucose transport and metabolism in adipocytes from patients with NIDDM immediately raises the possibility that these changes play a role in the insulin resistance that characterizes these individuals. Evidence in support of this view can be derived from the presence of significant correlations between the magnitude of hyperglycemia and the decreases in maximal insulin-stimulated glucose transport and utilization present in adipocytes from patients with NIDDM. Since previous studies in diabetic humans have indicated that a close correlation also exists between decreases in insulin-stimulated glucose uptake in patients with NIDDM and the severity of fasting hyperglycemia (34), there is considerable temptation to conclude that the changes we observed in insulin-stimulated glucose uptake and metabolism in adipocytes from patients with NIDDM account for the in vivo insulin resistance observed in these subjects. However, it must be emphasized that it would be premature to come to this conclusion in light of available data. In the first place, adipose tissue only accounts for a small proportion of total body glu-

[3] After submission of the current manuscript these studies have been published. Bolinder, J., J. Ostman, and P. Arer. 1982. Postreceptor defects causing insulin resistance in normo-insulemic non-insulin-dependent diabetes mellitus. *Diabetes*. 31:911–916.

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TABLE III
Insulin Binding

Subjects	n	Insulin binding per cell	Insulin binding per cell surface area
		pl/cell	*fl/μm²*
Control	7	192±26	4.1±0.5
NIDDM	7	156±24	3.8±0.5

All data are expressed as mean±SEM.

cose utilization (35). Thus, the defects noted in this study can only account for in vivo insulin resistance if similar abnormalities are present in other tissues, e.g., liver and/or muscle. In addition, it is important to emphasize that the fact that two variables are correlated does not prove that they are causally related. Furthermore, considerable variation in maximal insulin-stimulated glucose transport and utilization was noted in the age- and weight-matched group of subjects with normal glucose tolerance studied simultaneously, and defects in adipocyte glucose metabolism could be found in some of these normal individuals, which were comparable in magnitude to defects seen in adipocytes obtained from patients with NIDDM. Finally, even if the adipocyte defects and in vivo insulin resistance are causally linked, it does not mean that the in vitro tissue abnormalities are the cause of the insulin resistance. Experimentally induced insulin deficiency in dogs leads to the development of in vivo insulin resistance (36), and defects in adipocyte function similar to those described in this report have been documented in rats made insulin deficient (37, 38). Since patients with NIDDM can also be insulin deficient (3), it is possible that the abnormalities in adipocyte metabolism noted in this study could represent a secondary phenomenon. Finally, the decreased glucose uptake (in vivo and in vitro) is not necessarily the cause of the increased fasting plasma glucose concentrations in patients with NIDDM, especially since there is a real possibility that the liver may be the primary regulator of basal plasma glucose concentrations in these individuals. Obviously, ultimate decisions as to the role played by decreases in insulin-stimulated glucose transport and utilization by adipocytes from patients with NIDDM in the insulin resistance of this syndrome will depend upon results of future studies.

Although it is clearly a mistake to overinterpret the significance of the current data as to the etiology of NIDDM, it would be equally inappropriate to ignore the potential impact of these findings. Consequently, it seems reasonable to at least comment upon the insights provided by the data we have presented. In the first place, our results documented a decline in both the sensitivity and the capacity of the glucose transport system in adipocytes from patients with NIDDM. In contrast, there was no change in the relative responsiveness of the transport system, i.e., the percent to which insulin-stimulated glucose transport increase

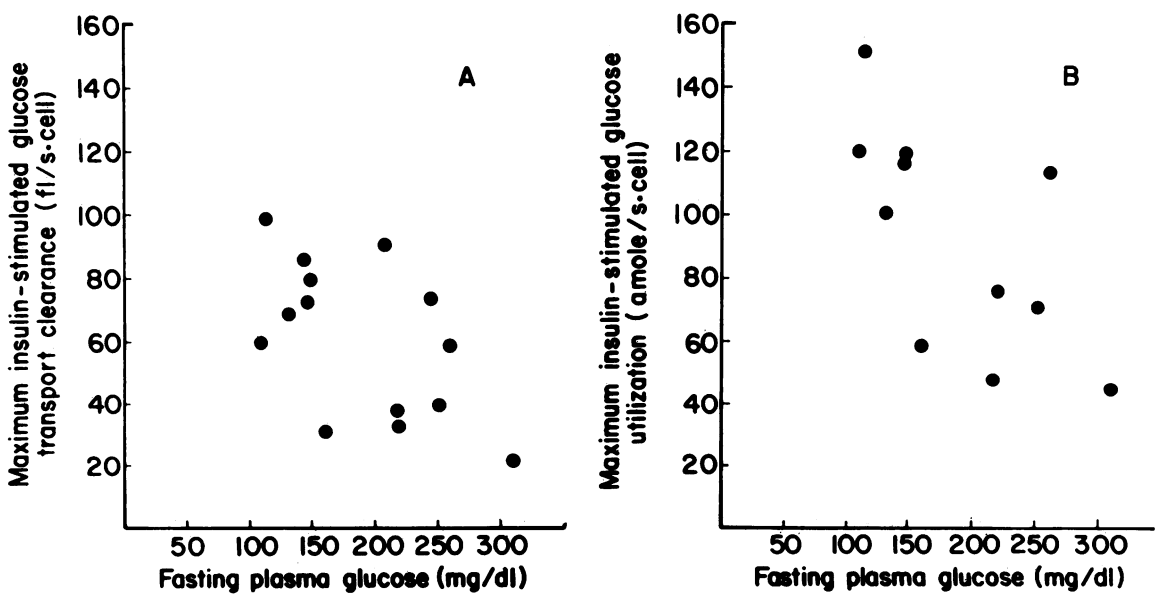

FIGURE 5 Correlation between fasting plasma glucose and maximum insulin-stimulated glucose transport (*A*) ($r = -0.56$, $P < 0.05$) and utilization (*B*) ($r = -0.67$, $P < 0.05$) in adipocytes isolated from NIDDM subjects (●).

was similar in adipocytes from age- and weight-matched normal subjects and patients with NIDDM. In other words, the sensitivity and the capacity were reduced, but the ability of the transport system to respond to insulin seemed unchanged. Decreases in insulin sensitivity can theoretically occur as a result of a reduction in insulin binding (4, 7, 23), but this does not appear to account for the findings in the current study (Table III). Our inability to document a reduction in insulin binding by adipocytes of patients with NIDDM appears to be at variance with the recently published data of Kolterman et al. (39). However, it should be pointed out that these authors did not, as we have done, directly compare insulin binding of adipocytes from obese patients with NIDDM with adipocytes isolated from equally obese subjects with normal glucose tolerance. Since it is known that adipocyte insulin binding is reduced in obese normal subjects (39), this difference in control group may account for the variation between our results and theirs. In any event, we cannot attribute the reduction in insulin sensitivity of the glucose transport system seen in adipocytes of patients with NIDDM to a change in insulin binding. Consequently, we must postulate that the observed reduction in both insulin sensitivity and capacity of the glucose transport system are secondary to postbinding defects.

The observation that there were parallel changes in both maximal insulin-stimulated glucose transport and utilization is also consistent with the notion that intracellular defects are responsible for abnormalities in adipocyte glucose metabolism seen in patients with NIDDM. Thus, defects in maximal insulin-stimulated glucose utilization were noted in studies carried out at a glucose concentration of 5.5 mM. As indicated in the Methods section, glucose utilization, not glucose transport, is rate limiting in this situation. Therefore, the defect in maximal insulin-stimulated glucose utilization must be viewed as being a primary abnormality, and not as a secondary consequence of the reduction in insulin-stimulated glucose transport. These considerations suggest the presence of widespread abnormalities, affecting both glucose transport and utilization, in adipocytes from patients with NIDDM. This view receives further support from the fact that all pathways of intracellular glucose catabolism were comparably reduced in adipocytes from diabetic subjects. Therefore, it seems most reasonable to suggest that NIDDM leads to a general decline in protein synthesis of both the transporters and the intracellular enzymes that regulate adipocyte glucose utilization. Obviously, this formulation is quite speculative, but it has the advantage of being amenable to direct experimental verification.

In conclusion, the results of the current studies document a reduction in both the sensitivity and the capacity of the glucose transport system, of adipocytes from patients with NIDDM, associated with a parallel decrease in maximal insulin-stimulated glucose utilization. These changes were related to the magnitude of the hyperglycemia in these patients, could not be attributed to a decrease in insulin binding, and seemed to be secondary to widespread defects in intracellular glucose metabolism. The significance of these findings in the pathogenesis of NIDDM remains to be clarified, and it must be remembered that these studies were carried out on Pima and Papago Indians. Consequently, it is possible that the changes in function of adipocytes isolated from the patients with NIDDM are unique to this population. However, the fact that preliminary reports have described somewhat similar findings in population characterized by genetic heterogeneity makes this possibility less likely (30–32).[3] Thus, it seems reasonable to assume that important information concerning the pathogenesis of NIDDM will evolve from further definition of the relationship between the tissue changes in adipocyte metabolism, in vivo insulin resistance, and the magnitude of the diabetic state in Pima Indians with NIDDM.

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